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DATA EVALUATION RECORD

NAPHTHALENE
NONGUIDELINE
STUDY TYPE: FOUR-WEEK INHALATION STUDY IN RATS
MRID 42934901

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
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Prepared by

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Task Order No. 171-2007

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DATA EVALUATION RECORD

STUDY TYPE: Subchronic Inhalation Toxicity - Rat; Nonguideline

PC CODE: 005801 **DP BARCODE:** D335943

TEST MATERIAL (PURITY): Naphthalene (assumed pure)

SYNONYMS: Camphor tar; Mothballs; Moth Flakes; Naphthene; tar camphor; white tar

CITATION: Coombs, D.W. (1993). Naphthalene; 4-week inhalation study in rats. Huntingdon Research Centre, Ltd., P.O. Box 2, Huntingdon, Cambridgeshire, England, PE18 6ES. Laboratory ID: LDA 1/921559. August 4, 1993. MRID 42934901. Unpublished.

SPONSOR: Landis International, Inc., P.O. Box 5126, Valdosta, GA 31603-5126

EXECUTIVE SUMMARY:

In a four-week inhalation dose-range finding study (MRID 42934901), groups of five male and female Sprague Dawley rats were exposed nose-only to 0, 0.005, 0.016, 0.052, 0.157, or 0.404 mg/L concentrations of naphthalene (purity not reported; (Lot No. LI-1 LX No. LX158-01) six hours/day, five days/week for four weeks.

No clinical signs of toxicity were observed and no deaths were reported during the study. Treatment related decreases in body weight and food intake were observed although these were unrelated to exposure concentration. No clinically significant effects were noted on measured hematology or clinical chemistry parameters and no treatment-related effects were noted at necropsy. No effects on organ weights were found.

Treatment and dose-related effects were noted in the nasal turbinates. These included slight disorganization, rosette formation, basal cell hyperplasia, erosion, atrophy, and degenerate cells in the olfactory epithelium, loss of Bowman's glands, hypertrophy of respiratory epithelium, rosette formation in the septal organ of Masera and fusion of the turbinates. These effects were dose-related and more noticeable in the Intermediate, Mid-High, and High exposure groups with only minor changes observed in the Low and Mid-Low exposure groups. In addition, one female rat in the High-exposure group developed lenticular degeneration. This type of lesion rarely occurs spontaneously and is likely related to exposure.

The LOAEL for naphthalene in male and female Sprague Dawley rats is 0.052 mg/L based on the increased incidence and severity of changes to the nasal turbinates. The NOAEL for male and female Sprague Dawley rats is 0.016 mg/L.

This inhalation toxicity study in the rat is considered **Acceptable/Nonguideline.**

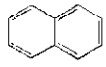
COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS:

A. MATERIALS:

1. Test material: Naphthalene

Description: Grey-white crystalline solid
Lot/batch #: LI-1 (LX No. LX158-01
Purity: Assumed pure
Compound stability: Not reported
CAS # of TGAI: 91-20-3
Structure:



2. Test animals:

Species: Rat
Strain: Sprague-Dawley
Age/weight at study initiation: Approximately six weeks. Males 268-329 g; Females 203-253 g
Source: Charles River Limited, Portage, MI
Housing: Groups of five/sex/exposure concentration in suspended stainless steel cages with mesh floors
Diet: SDS Rat and Mouse No.1, *ad libitum* (except during exposure) from weighed amounts
Water: Tap water, *ad libitum*
Environmental conditions:
Temperature: 18-27.5EC
Humidity: 40-80%
Air changes: Not reported
Photoperiod: 12 hrs light/dark
Acclimation period: Approximately one week

B. STUDY DESIGN:

1. In life dates: Start: July 20, 1992; End: August 16-17, 1992

2. Animal assignment: Animals were randomly assigned based on body weight to the test groups noted in Table 1.

TABLE 1: Study design

Test group	Target conc. (mg/L)	Analytical conc. (mg/L)	Percent of nominal	MMAD and GSD	Rats/sex
Control	0	0	100	NA	5
Low	0.005	0.005	100	NA	5
Mid Low	0.016	0.017	106	NA	5
Intermediate	0.052	0.055	106	NA	5
Mid High	0.157	0.153	97	NA	5
High	0.404	0.372	92	NA	5

Data from page 21, MRID 42934901

3. **Dose selection rationale:** The study was done to establish exposure concentrations for a subsequent 90-day study.

4. **Generation of the test atmosphere / chamber description:** Separate vapor generation systems were used for each exposure concentration. Each system was composed of a three-necked round bottom flask that contained an aliquot of the test material. Air was passed into the flask through one neck and the vapor-laden air passed out of the flask through a second neck. The third neck of the flask contained a thermometer to monitor generation temperature. At lower exposure concentrations, the test atmosphere was generated under ambient conditions using carrier air flow to maintain exposure concentration. At exposure concentrations ≥0.052 mg/L, the generation flask and carrier air were heated in a water bath to facilitate vaporization. The vapor-laden air was passed through a clear plastic tube packed with glass wool as a particle trap. The vapor was mixed with diluent air (when applicable) before entering the nose-only exposure system. All airflows were monitored by in-line rotameters. During each exposure, the rats were restrained in molded polycarbonate tubes tapered at one end to allow only the snout to protrude into the exposure chamber. Air entered the chamber at a flow rate of 25 L/min and was exhausted from the base of the chamber at 30 L/min. This maintained a slight negative pressure inside the chamber to prevent leakage of the exposure atmosphere. Rats in all groups were exposed to the test atmosphere 6 hours/day, 5 days/ week, for four weeks.

   Time to 90% equilibrium was four minutes.

   The concentration of naphthalene in the exposure atmosphere was measured 1, 3, and 5 hours after the start of each exposure. The concentration of test material was measured by drawing a known volume of exposure atmosphere through a charcoal adsorption tube. The contents of the tube were extracted into a known volume of carbon disulfide for twenty minutes. The extraction solution was placed into glass chromatography vials and analyzed by flame-ionization gas chromatography. With this system, the retention time of naphthalene was ~1.6 minutes. Results are in Table 1 above.

   The determination of MMAD and GSD were not relevant to the study.

5. **Statistics:** If greater than 75% of the values were identical within a treatment group, frequency analysis was used. Otherwise, the data were evaluated for homogeneity using Bartlett's test. Data with homogenous variances were analyzed by ANOVA followed by Student's 't' test and Williams' test. If the data were non-homogenous, a logarithmic transformation was used to stabilize the variance. If the data were heterogeneous after transformation, the untransformed data were analyzed using the Kruskal-Wallis test followed by the Shirley test. Organ weight data were analyzed using analysis of covariance, using terminal body weight as the covariate.

C. METHODS:

1. Observations:

1a. Cageside observations: All rats were examined twice daily for morbidity and moribundity throughout the study.

1b. Clinical examinations: Clinical examinations were conducted at weekly intervals during the study.

1c. Neurological evaluations: Neurological evaluations were not done.

2. Body weight: Animals were weighed weekly.

3. Food consumption: The rats were housed in groups of five rats/sex/exposure concentration. Food consumption for each cage of rats was recorded weekly by subtracting the initial from final food weight.

4. Ophthalmoscopic examination: The eyes were not examined.

5. Hematology and clinical chemistry: Blood was collected during Week 4 from the orbital sinus of anesthetized rats. EDTA and sodium citrate were used as anticoagulants for the hematological studies and lithium heparin was used as the anticoagulant for the clinical chemistry studies. The CHECKED (X) parameters were examined.

a. Hematology:

X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*		Mean corpuscular HGB (MCH)*
X	Leukocyte count (WBC)*	X	Mean corpusc. HGB conc.(MCHC)*
X	Erythrocyte count (RBC)*	X	Mean corpusc. volume (MCV)*
X	Platelet count*	X	Reticulocyte count
	Blood clotting measurements*		
	(Thromboplastin time)		
	(Clotting time)		
X	(Prothrombin time)		

* Recommended for subchronic inhalation studies based on Guideline 870.3465

b. Clinical chemistry:

X	ELECTROLYTES	X	OTHER
X	Calcium	X	Albumin*
X	Chloride	X	Creatinine*
	Magnesium	X	Urea nitrogen*
X	Phosphorus	X	Total Cholesterol*
X	Potassium*	X	Globulins
X	Sodium*	X	Glucose*
X	**ENZYMES** (more than 2 hepatic enzymes eg., *)	X	Total bilirubin
X	Alkaline phosphatase*	X	Total serum protein (TP)*
	Cholinesterase (ChE)		Triglycerides
X	Creatine phosphokinase		Serum protein electrophoresis
	Lactic acid dehydrogenase (LDH)		
X	Alanine aminotransferase (ALT/also SGPT)*		
X	Aspartate aminotransferase (AST/also SGOT)*		
	Sorbitol dehydrogenase*		
X	Gamma glutamyl transferase (GGT)*		
	Glutamate dehydrogenase		

* Recommended for subchronic inhalation studies based on Guideline 870.3465

6. **Urinalysis**: Urinalysis was not done.

7. **Sacrifice and pathology:** All animals that died and those sacrificed on schedule were subjected to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. All tissues were examined microscopically from control and high-exposure group rats. The eyes and nasal passages from rats in all groups were also examined. The (XX) organs, in addition, were weighed.

X	DIGESTIVE SYSTEM
	Tongue
	Salivary glands*
	Esophagus*
	Stomach*
	Duodenum*
	Jejunum*
	Ileum*
	Cecum*
	Colon*
	Rectum*
XX	Liver*+
	Gall bladder* (not rat)
	Bile duct* (rat)
	Pancreas*
X	**RESPIRATORY**
X	Trachea*
XX	Lung*
X	Nose* (rostral and caudal cavities)
X	Pharynx*
X	Larynx*

X	CARDIOVASC./HEMAT.
X	Aorta, thoracic*
X	Heart*+
	Bone marrow*
X	Lymph nodes*
X	Spleen*+
	Thymus*+
X	**UROGENITAL**
XX	Kidneys*+
	Urinary bladder*
XX	Testes*+
XX	Epididymides*+
	Prostate*
	Seminal vesicles*
	Ovaries*+
	Uterus*+
	Mammary gland*

X	NEUROLOGIC
	Brain*+
	Peripheral nerve*
	Spinal cord (3 levels)*
	Pituitary*
X	Eyes (optic nerve)*
X	**GLANDULAR**
XX	Adrenal gland*+
	Lacrimal gland
	Parathyroid*
	Thyroid*
X	**OTHER**
	Bone (sternum and/or femur)
	Skeletal muscle
	Skin
X	All gross lesions and masses*

* Recommended for subchronic rodent studies based on Guideline 870.3465
+ Organ weights required

II. RESULTS:

A. OBSERVATIONS :

1. **Clinical signs of toxicity:** No treatment-related clinical signs of toxicity were observed during exposure or at other times during the study.

2. **Mortality:** All rats survived until study termination.

B. BODY WEIGHT AND WEIGHT GAIN:

The body weight gain of both sexes in all exposure groups was decreased relative to control by the end of the study. Although a dose-response was not evident in males, the body weight gain was statistically decreased to the greatest extent in the Intermediate, Mid-high, and High exposure groups.

TABLE 2. Average body weight (g) and body weight gain (g) of rats exposed to naphthalene for four weeks								
Group (mg/L)	**Week -1**	**Week 0**	**Week 1**	**Week 2**	**Week 3**	**Week 4**	**Total Wt. Gain (g)**	**Percent Difference from Control**
Male								
Control (0)	253	311	339	358	382	401	90	--
Low (0.005)	244	292	307	328	349	358	66	-27
Mid-Low (0.016)	252	307	331	344	363	377	70	-22
Intermediate (0.052)	243	302	312	320	337	344	42**	-53
Mid-High (0.157)	251	307	319	329	347	357	50**	-44
High (0.404)	250	311	320	333	348	357	46**	-49
Female								
Control (0)	188	221	231	239	248	260	39	--
Low (0.005)	194	226	233	234	250	258	32	-18
Mid-Low (0.016)	190	223	228	235	241	247	24*	-38
Intermediate (0.052)	195	228	229	236	248	250	22**	-44
Mid-High (0.157)	191	217	224	225	235	238	21**	-56
High (0.404)	193	221	223	223	231	239	18**	-54

Data from pages 45 and 46 of MRID 42934901
* $p \leq 0.05$
** $p \leq 0.01$

C. FOOD CONSUMPTION:

Food consumption: Total food consumption was generally decreased in all exposure groups, although a dose-response relationship was not established. Total food consumption was decreased relative to control 10, 7, 15, 15, and 14% in male rats and 0, 6,, 10, 11, and 19%, in female rats of the Low, Mid-Low, Intermediate, Mid-High, and High exposure groups, respectively.

D. BLOOD ANALYSES:

1. **Hematology:** No toxicologically relevant effects were noted in hematological parameters. Although the RBC count of high dose females and the Thrombotest of high-dose males were statistically increased, the changes were minor and not of biological significance.

2. **Clinical chemistry:** Although random statistically significant effects were noted, none were of sufficient magnitude to be biologically or toxicologically relevant.

E. SACRIFICE AND PATHOLOGY:

1. **Organ weight:** Although the absolute covariant liver weight of High-exposure females was statistically increased, the increase was <8% and was not toxicologically or biologically relevant. No other treatment-related effects were noted.

2. **Gross pathology:** No toxicologically significant macroscopic effects were noted at necropsy.

3. **Microscopic pathology:** The only significant effects of inhalation exposure to naphthalene were found in the nasal turbinates (Table 3). The effects were dose-related and more noticeable in the Intermediate, Mid-High, and High exposure groups with only minor changes

observed in the Low and Mid-Low exposure groups. In addition, one female rat in the High-exposure group developed lenticular degeneration with proliferation of the subcapsular cells. This type of lesion rarely occurs spontaneously, and is likely related to exposure.

TABLE 3. Incidence of treatment-related effects to nasal turbinates of rats exposed to naphthalene 6 hours/day, 5 days/week for four weeks.

Effect	Males						Females					
	CT	L	ML	I	MH	H	CT	L	ML	I	MH	H
Number examined	5	5	5	5	5	5	5	5	5	5	5	5
No abnormalities	5	1	0	0	0	0	4	1	0	0	0	0
Occasional epithelial degeneration	0	0	2	4	2	5	0	1	5	5	3	5
Epithelial erosion	0	0	0	0	0	2	0	0	0	0	2	0
Epithelial atrophy	0	0	0	0	1	0	0	0	0	0	0	0
Slight epithelial disorganization	0	1	0	0	0	0	0	3	0	0	0	0
Epithelial rosette formation	0	2	5	5	5	5	0	4	5	5	5	5
Rosette formation in septal organ of Masera	0	2	0	0	0	0	0	0	0	0	0	0
Hyperplasia of basal epithelial cells	0	0	5	5	5	5	0	0	5	3	4	5
Loss of Bowman's glands	0	0	0	3	5	4	0	0	0	2	4	5
Fusion of turbinates	0	0	0	0	0	1	0	0	0	0	0	0
Respiratory epithelial hypertrophy	0	0	0	2	2	0	0	0	1	0	1	1
Hyperplasia of primitive cells in Bowman's glands	0	0	0	2	1	1	0	0	0	0	0	0
Dilated gland duct	0	0	0	1	0	0	0	0	0	1	0	0

Data from pages 57 and 61 of MRID 42934901.

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATORS= CONCLUSIONS:

Based on the results, the study author concluded that exposure of male and female rats to naphthalene vapor six hours/day, five days/week for four weeks reduced the body weight and food intake. No macroscopic changes were noted at necropsy; however dose-related effects were noted in the nasal turbinates. In addition, one female rat in the high-exposure group developed lenticular degeneration with proliferation of the subcapsular cells that was likely related to naphthalene exposure.

B. REVIEWER COMMENTS:

In this study, groups of five male and five female rats were exposed six hours/day, five days/week for four weeks to naphthalene. No clinical signs of toxicity were observed and no deaths were reported during the study. Treatment related decreases in body weight and food intake were observed although these were unrelated to exposure concentration. No clinically significant effects were noted on measured hematology or clinical chemistry parameters. No treatment-related effects were noted macroscopically at necropsy and no effects on organ weights were found.

Treatment and dose-related effects were noted in the nasal turbinates. These included slight disorganization, rosette formation, basal cell hyperplasia, erosion, atrophy, and degenerate cells in the olfactory epithelium, loss of Bowman's glands, hypertrophy of respiratory epithelium, rosette formation in the septal organ of Masera and fusion of the turbinates.

These effects were dose-related and more frequent in the Intermediate, Mid-High, and High exposure groups with only minor changes observed in the Low and Mid-Low exposure groups. In addition, one female rat in the High-exposure group developed lenticular degeneration. This type of lesion does not occur spontaneously, and is likely related to exposure.

Based on the results, the **LOAEL** for naphthalene in male and female Sprague-Dawley rats is 0.052 mg/L based on the increased incidence and severity of lesions found in the nasal turbinates. The corresponding **NOAEL** is 0.016 mg/L.

C. STUDY DEFICIENCIES:

Some pages of the study report were missing, while multiple copies of other pages were included in the study report. The purity of the naphthalene used in the study was not reported.

13544

R154936

Chemical:

PC Code:

HED File Code: 258 Contractor DER TOX Warning: This Document (review) was completed by a contractor and has not undergone secondary review. This document may not reflect Agency policies

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